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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 99 (FGE.99):

Consideration of furanone derivatives evaluated by the JECFA (63rd, 65th and 69th meetings)¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of five furanone derivatives evaluated by the JECFA at their 63rd, 65th and 69th meetings. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for one of the five substances [FL-no: 13.010]. Contrary to the JECFA the Panel allocate three substances [FL-no: 13.084, 13.085 and 13.099] to structural class III due to lack of information on natural occurrence. With regards to the substance [FL-no: 13.176] for which the JECFA concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied because of the unresolved toxicological concerns relating to the epoxidation and opening of the furan ring, the Panel concluded that adequate NOAELs exist and accordingly concluded, “No safety concern at the estimated level of intake”. Therefore, the Panel concluded that all five substances do not give rise to safety concern at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these

1 On request from the Commission, Question No EFSA-Q-2007-00577, EFSA-Q-2007-00594, EFSA-Q-2007-00595, EFSA-Q-2007-00597 and EFSA-Q-2007-00608 adopted on 27 September 2012.

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flavouring substances, the specifications for the materials of commerce have also been considered and for all five substances, the information is adequate.

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KEY WORDS

Furanone derivatives, flavourings, food safety, JECFA, 63rd meeting, 65th meeting, 69th meeting, FGE.99

SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

This consideration deals with five furanone derivatives, 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010], 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084], 4-hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085], 4-acetoxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.099] and furan-2-yl butyrate [FL-no: 13.176], which are in the Register and which were evaluated by the JECFA at its 63rd, 65th and 69th meetings.

The Panel concluded that no supporting Flavouring Group Evaluation (FGE) was available for the substances in the present FGE.

The Panel agrees with the application of the Procedure as performed by the JECFA for only one substance [FL-no: 13.010] considered in this FGE. The JECFA stated that the three substances [FL-no: 13.084, 13.085 and 13.099] belong to structural class II, but as data on natural occurrence are not available for these three substances, the Panel disagrees with this and allocates these three substances to structural class III. In addition, for the substance [FL-no: 13.176] the JECFA concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied because of the unresolved toxicological concerns relating to the epoxidation and opening of the furan ring. The Panel disagreed with the conclusions by the JECFA and regarded this substance to be sufficiently structurally related to the other substances in this group.

The Panel concluded that there is no safety concern for the candidate substances in this FGE, based on the MSDI approach.

For all substances evaluated through the Procedure use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all the JECFA evaluated substances.

Thus, for all five substances [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] the Panel concluded that they would be of “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 - 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments. The evaluation programme was finalised at the end of 2009.

The Commission requested EFSA, based on additional submitted data on genotoxicity, to carry out a re-evaluation of the flavouring substance, 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010], and depending on the outcome, to proceed to the evaluation of these flavouring substances through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “JECFA Procedure”. The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA at its 65th meeting considered “how to improve the identification and assessment of flavouring agents for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods” (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

“The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure (“Do the condition of use result in an intake greater than 1.5 microgram per day?”) (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999a), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

The five furanone derivatives [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] were originally members of a group of 58 substances evaluated by the JECFA at their 63rd, 65th and 69th meetings (JECFA, 2005c; JECFA, 2006b; JECFA, 2009c) and discussed by EFSA in FGE.75 (EFSA, 2008aw). The Panel concluded that these five substances are structurally different to the other substances in FGE.75 and therefore these five substances will be considered in a separate FGE (FGE.99). As the five candidate substances are α,β -unsaturated ketones they have been considered together with other α,β -unsaturated substances with respect to genotoxicity in FGE.220Rev1 (EFSA, 2011a). The Panel concluded that the data available ruled out the concern for genotoxicity and thus concluded that these five substances can be evaluated through the Procedure.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has at the 63rd meeting evaluated a group of 18 flavouring substances consisting of tetrahydrofuran and furanone derivatives (JECFA, 2005c).

The JECFA has at the 65th meeting evaluated a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulphides, disulphides and ethers (JECFA, 2006b) where a request for additional data was expressed. The furan group was on the agenda again at the 69th JECFA meeting (JECFA, 2009c) where additional data had been provided.

1.1.2. EFSA Considerations

This FGE only deals with five of the above mentioned 58 substances, four substances evaluated by the JECFA at the 63rd meeting [FL-no: 13.010, 13.084, 13.085, 13.099] and one substance evaluated by the JECFA at the 65th meeting [FL-no: 13.176]. They are all furanone derivatives and the Panel concluded that these substances should be considered in a separate group.

Accordingly five substances [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] will be considered in the present FGE.

1.2. Isomers

1.2.1. Status

Four substances in the present group of furanone derivatives have one chiral centre.

1.2.2. EFSA Considerations

Adequate information on isomeric composition is available for all the candidate substances in FGE.99.

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all five substances (JECFA, 2005b; JECFA, 2005d) (see Table 1).

1.3.2. EFSA Considerations

The available specifications are considered adequate for all five candidate substances in FGE.99 (See Section 1.2 and Table 1).

2. Intake Estimations

2.1. JECFA Status

For all substances evaluated through the JECFA Procedure, intake data are available for the EU.

2.2. EFSA Considerations

Tonnage data are available for the EU allowing calculation of the intake estimates (MSDI). The Panel noted that since no use levels were submitted no mTAMDI values can be calculated.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken⁴ from the JECFA (JECFA, 2006a)

In vitro

4-Hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] (10 - 12,000⁵ µg/plate), 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] (10 - 10,000 µg/plate), and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] (up to 10,000 µg/plate) induced reverse mutations in standard and modified Ames assays. Positive results were obtained for 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] in *Salmonella typhimurium* strains TA100, TA102, TA98 and TA97 at the highest dose tested (4000 µg/plate) with or without metabolic activation (Xing et al., 1988). In contrast, (Gilroy et al., 1978) and (Hiramoto et al., 1996b) reported positive results for this compound only in *S. typhimurium* strain TA100 when tested at concentrations of ≤ 10,000 µg/plate with or without metabolic activation. Similarly, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] and 4-hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] produced positive results in *S. typhimurium* strain TA100 with or without metabolic activation (Hiramoto et al., 1996a; Li et al., 1998).

The standard Rec assay with *Bacillus subtilis* H17 (rec+) and M45 (rec-) exposed to 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] at a concentration of 20, 40, 60, 80 and 120 µg/disc yielded a dose-dependent DNA damage response (Xing et al., 1988).

⁴ The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

⁵ Maximum concentration tested with [FL-no: 13.085] could not be verified in the publications cited by JECFA (2006a). These publications mention a maximum concentration of 5000 µg/plate, which has been mentioned in the tables in this FGE.

In vivo

In an assay for genotoxicity *in vivo*, groups of five ICR mice were given a negative control, or 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] or 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] at a concentration of 1000, 2000 or 3000 mg/kg bw by oral administration. Blood was drawn at intervals of 15 min. after administration for up to 120 min. For 4-hydroxy-2,5-dimethylfuran-3(2H)-one, the frequency of micronucleated peripheral reticulocytes was increased at a dose of 2000 and 3000 mg/kg bw, but not at 1000 mg/kg bw. For 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone, the frequency was increased at all three doses (Hiramoto et al., 1998). Kunming mice injected intraperitoneally with 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] at a dose of 0, 186, 232 or 309 mg/kg bw demonstrated a dose-dependent increase in erythrocyte micronucleus formation in the bone marrow, reaching a maximum increase of 2.6-fold (Xing et al., 1988). Also male mice injected with 4-hydroxy-2,5-dimethylfuran-3(2H)-one at a dose of 0, 232, 464 or 928 mg/kg bw exhibited increases in spermatocyte chromosome aberrations (Xing et al., 1988). In a similar assay in male Kunming mice given 4-hydroxy-2,5-dimethylfuran-3(2H)-one at a dose of 200, 400 or 800 mg/kg bw by intragastric instillation, a significant increase in sister chromatid exchanges in spermatogonial cells compared to controls was reported at all three doses (Tian et al., 1992). Positive results were also obtained in an assay for micronucleus formation in male mice given 4-hydroxy-2,5-dimethylfuran-3(2H)-one at a dose of 200, 400 or 800 mg/kg bw by intraperitoneal injection (Tian et al., 1992). The increases observed at each dose did not establish a clear dose-response relationship, although increases were significantly higher than for the negative control.

Groups of five or six male ICR mice were given 4-hydroxy-2,5-dimethylfuran-3(2H)-one at a dose of 0, 500, 1000 or 1500 mg/kg bw by intraperitoneal injection. Blood samples were drawn at 24, 48 and 72 hours after injection. The frequency of micronucleated peripheral erythrocytes was significantly increased at ≥ 500 mg/kg bw, with the maximum frequency of 1.6 % being obtained at 48 hours after dosing (Hiramoto et al., 1996b).

Groups of five or six male ICR mice were given 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] at a dose of 500, 1000 or 1500 mg/kg bw by intraperitoneal injection and samples of peripheral blood were taken at 24, 48 and 72 hours after injection. All the mice in the group given 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone at a dose of 1500 mg/kg bw died before 24 hours. The frequency of micronucleated peripheral erythrocytes was significantly higher than that in the controls in groups given 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone at a dose of 500 or 1000 mg/kg bw. The maximum number of micronucleated peripheral erythrocytes was observed at 48 hours at 1000 mg/kg bw group (0.58 %) and at 500 mg/kg bw (approximately 0.3 %). The frequency of micronucleated peripheral erythrocytes reported in mice given the positive control substance, mitomycin C, at a dose of 1 mg/kg bw, was 3.1 % (Li et al., 1998).

In summary, positive results were obtained in several assays for genotoxicity *in vivo* in mice given 4-hydroxy-2,5-dimethylfuran-3(2H)-one via intraperitoneal injection at doses as low as 196 mg/kg bw (Xing et al., 1988). Similarly, positive results were also obtained for 4-hydroxy-2,5-dimethylfuran-3(2H)-one administered orally; however, there are conflicting data pertaining to the lowest dose at which 4-hydroxy-2,5-dimethylfuran-3(2H)-one elicits a positive response: 200 mg/kg bw according to (Tian et al., 1992); ≥ 2000 mg/kg bw according to (Hiramoto et al., 1998).

Putative mechanism of genotoxicity of furanone derivatives

Furanones induce DNA damage *in vitro* by generating free radicals that induce strand scission. In the presence of metals (e.g. Fe^{3+}) and dissolved oxygen, the enolic hydroxyl group (OH) of the furanone is oxidized by single electron transfer to yield the corresponding carbon-centred radical and a reduced metal ion (e.g. Fe^{2+}). The carbon-centred radical can couple to molecular oxygen to produce a peroxy radical that may damage DNA. Alternately, the reduced metal ion can auto-oxidize to form a superoxide radical anion. The superoxide radical then dismutates into hydrogen peroxide (H_2O_2). It is well recognized that reduced metals react with H_2O_2 to form a hydroxyl radical, which is a powerful

oxidizing agent (see Figure 1). Hydrogen peroxide also oxidizes glutathione leading to decreased glutathione S-transferase/oxidized glutathione and an increase in cellular oxidative stress.

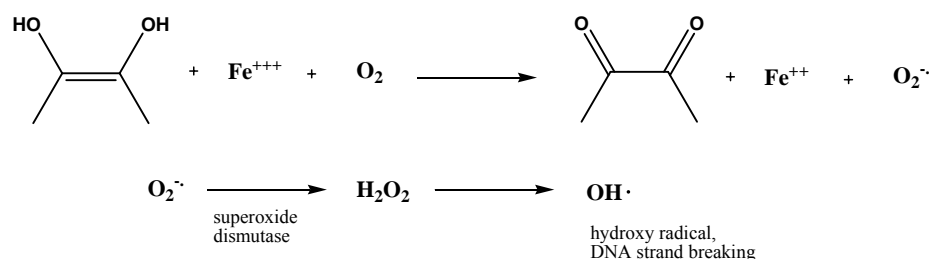


Figure 1 Mechanism of oxidation of furanone derivatives *in vitro*.

In the case of 4-hydroxy-2,5-dimethylfuran-3(2H)-one, experimental evidence for this H₂O₂-producing pathway includes the following:

- Fe³⁺ or Cu²⁺ is readily reduced to Fe²⁺ or Cu⁺ in the presence of 4-hydroxy-2,5-dimethylfuran-3(2H)-one;
- DNA strand-breaking of super coiled plasmid DNA into an open circular form in the presence of 4-hydroxy-2,5-dimethylfuran-3(2H)-one is inhibited in the presence of superoxide dismutase and catalase, enzymes used to detoxicate superoxide to form oxygen and water;
- hydroxyl radical scavengers such as potassium iodine, sodium azide, or ethanol also inhibit DNA strand-breaking;
- free radical spin-trapping agents (e.g. 5,5-dimethyl-1-pyrroline *N*-oxide) also inhibit DNA strand-breaking;
- oxygen radical trapping agents such as 2-mercaptoethanol and cysteine are also inhibitory;
- removal of dissolved oxygen by nitrogen purge decreases DNA strand-breaking and H₂O₂ formation;
- addition of metal chelating agents also inhibits DNA strand-breaking by depleting the metal ions required for this process;
- DNA strand-breaking by 4-hydroxy-2,5-dimethylfuran-3(2H)-one was much faster in the presence of Fe³⁺ than in its absence; and
- electron spin resonance of a solution of 4-hydroxy-2,5-dimethylfuran-3(2H)-one and 5,5-dimethyl-1-pyrroline *N*-oxide showed the presence of hydroxyl radicals and bicarbonate radicals (Hiramoto et al., 1996a; Hiramoto et al., 1996b; Yamashita et al., 1998).

On the basis of these observations, cellular oxidative stress is related to the dose-dependent oxidation of 4-hydroxy-2,5-dimethylfuran-3(2H)-one and structurally related furanones, yielding H₂O₂ and eventually hydroxyl radicals (Hiramoto et al., 1996a; Hiramoto et al., 1996b; Li et al., 1998; Yamashita et al., 1998).

The ability of 4-hydroxy-2,5-dimethylfuran-3(2H)-one to induce oxygen radical formation and DNA strand breaks is reminiscent of similar activities observed for vitamin C. Vitamin C (ascorbic acid) contains an enediol that is superficially related to the enol of 4-hydroxy-2,5-dimethylfuran-3(2H)-one. Being both an enol ether and an αβ-unsaturated ketone, 4-hydroxy-2,5-dimethylfuran-3(2H)-one is

subject to hydrolytic ring opening, to yield an enediol. Like 4-hydroxy-2,5-dimethylfuran-3(2H)-one, vitamin C also reduces metal ions and produces superoxide anions to generate hydroxyl radicals that cleave DNA. As anticipated, vitamin C exhibits genotoxicity in test systems similar to those in which furanones give positive results. In standard Ames assays, ascorbic acid (vitamin C) induces reverse mutations in *S. typhimurium* strains TA104, TA102, TA100 and TA98 at concentrations of 352 - 1761 µg/plate (Ichinotsubo et al., 1981b; D'Agostini et al., 2000). In the *E. coli* Mutoxitest, positive results were obtained when ascorbic acid at a concentration of 200, 300 or 400 µg/plate in the presence of Cu²⁺ was incubated with *E. coli* strain IC203 (Martinez et al., 2000). *E. coli* IC203 carries an *oxyR* mutation that effectively removes its ability to turn on the biosynthesis of H₂O₂-protective proteins and makes the strain sensitive to DNA damage under conditions of oxidative stress (Blanco et al., 1998).

Increased frequencies of micronucleus formation were observed when ascorbic acid (400, 500 or 600 µg/ml) was incubated with Chinese hamster cells (Miller et al., 1995). An increase in sister chromatid exchanges was observed in Chinese hamster ovary cells in the presence of ascorbic acid at 500 µg/ml without metabolic activation (Tennant et al., 1987). In a standard assay for micronucleus formation in mice, ascorbic acid at a dose of 1500 mg/kg bw induced a significant increase (Shelby et al., 1993).

Conclusion

Furanones are a class of substances present naturally in food and are also added as flavouring agents. The principal furanone used as a flavouring agent is 4-hydroxy-2,5-dimethylfuran-3(2H)-one. In humans, 4-hydroxy-2,5-dimethylfuran-3(2H)-one is rapidly absorbed in the gastrointestinal tract and conjugated with glucuronic acid in the liver. Free 4-hydroxy-2,5-dimethylfuran-3(2H)-one is not detected in the blood of human volunteers to whom it is administered as a constituent of strawberries; its glucuronic acid conjugate is the principal urinary metabolite (Roscher et al., 1997). Thus, the potential for chemical reaction of 4-hydroxy-2,5-dimethylfuran-3(2H)-one with important cellular macromolecules, especially DNA, appears to be low.

Genotoxicity with 3-(2H)-furanone derivatives, notably 4-hydroxy-2,5-dimethylfuran-3(2H)-one and 2-ethyl-4-hydroxy-5-methyl-3-(2H)-furanone, was observed in standardized bacterial (Gilroy et al., 1978; Xing et al., 1988; Hiramoto et al., 1996a; Hiramoto et al., 1996b; Li et al., 1998) and mammalian assays (Xing et al., 1988; Tian et al., 1992; Hiramoto et al., 1996b). A mechanism for genotoxicity involving dose-dependent formation of H₂O₂ and oxidized furanones has been extensively studied (Hiramoto et al., 1995; Hiramoto et al., 1996a; Hiramoto et al., 1996b); these studies indicate that, at high doses, DNA single-strand breaks result from the reaction of hydroxyl radicals with DNA.

Despite the fact that 4-hydroxy-2,5-dimethylfuran-3(2H)-one causes genotoxicity, it is not carcinogenic in rats. Two studies, one with 4-hydroxy-2,5-dimethylfuran-3(2H)-one and the other with a structurally related furanone, showed no evidence of carcinogenicity at intakes that are orders of magnitude greater than the intake of furanones added as flavouring agents (Munday and Kirkby, 1973; Kelly and Bolte, 2003). Furthermore, vitamin C, a structurally similar compound with a genotoxicity test profile similar to that of 4-hydroxy-2,5-dimethylfuran-3(2H)-one, does not demonstrate carcinogenicity (National Research Council, 1996). In a 2-year bioassay, the NOEL for 4-hydroxy-2,5-dimethylfuran-3(2H)-one was 200 mg/kg bw per day in rodents. This intake is approximately 2000 times higher than the daily per capita intake ('eaters only') of 0.088 mg/kg bw per day from use of 4-hydroxy-2,5-dimethylfuran-3(2H)-one as a flavouring agent.

After consideration of all the available data, the Committee concluded that it is highly unlikely that 4-hydroxy-2,5-dimethylfuran-3(2H)-one, other furanones or tetrahydrofurans, would pose any significant genotoxic risk to humans under the conditions of use as flavouring agents. Similarly, 2-ethyl-4-hydroxy-5-methyl-3-(2H)-furanone was considered not to pose a genotoxic risk.

EFSA Conclusion on genotoxicity assessment by the JECFA

The Panel notes the mechanism identified by the JECFA and agrees that the positive results obtained in genotoxicity studies for these studies are likely to be due to the formation of reactive oxygen species. However, the Panel concluded that the possibility of genotoxicity in germ cells had not been excluded and in FGE 220 (EFSA, 2009ae), the Panel requested further studies from Industry on germ cell genotoxicity. Further studies were submitted by Industry and reviewed by the panel in FGE. 220Rev 1 (EFSA, 2011a). The Panel's conclusions on these studies can be found in Section 3.2.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by the JECFA, see Table 2.1 and 2.2.

There is no relevant text on genotoxicity from the 65th and 69th meetings on the substances in question.

3.2. Genotoxicity Studies and Conclusion on Genotoxicity and Carcinogenicity - Text taken⁶ from FGE.220Rev1 (EFSA, 2011a).

The following text is relevant for all five substances [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] in this FGE.

Subgroup 4.4b (Furan-3(2H)-ones in which the α,β -double bond can be involved in keto-enol tautomerism).

For three substances, which belong to subgroup 4.4b the following results have been reported:

4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010]

For 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] publications on *in vitro* and *in vivo* studies are available. In three studies the potential of the test substance to induce gene mutations in *S. Typhimurium* was studied. The substance was found positive in two valid studies and in one study with limited validity. The substance did not cause gene mutations in a valid study in *Escherichia coli* WP2 uvrA⁻. It was also observed that the substance caused DNA repair in a less relevant bacterial test and single strand breaks in purified DNA.

All *in vivo* studies provided indications for a genotoxic potential. Two studies showing micronucleus formation in peripheral blood cells were considered valid (Hiramoto et al., 1996b; Hiramoto et al., 1998); in a third study similar evidence but of limited validity was obtained (Xing et al., 1988). The latter authors also reported an increase in sister chromatid exchanges (SCE) in mouse bone marrow, but the validity of that observation could not be assessed. In addition this endpoint is of questionable relevance for the assessment of genotoxicity.

In addition to the genotoxicity observed in somatic cells, three studies provided evidence for genotoxicity in germ cells.

The evidence of chromosome aberration induction in mouse germ cells provided in the study by Xing et al. (1988) is poor because it is essentially based on an increase of premature disjunction of sex chromosomes and autosomes at metaphase I. This effect could be considered at most an alert of possible subsequent missegregation events; even so, data have been published (Liang and Pacchierotti, 1988) showing the lack of correlation between univalents at metaphase I and aneuploidy at metaphase II.

Tian et al. (1992) reported an induction of SCE in spermatogonia. Incomplete information is given on the experimental protocol. There is a dose-dependent increase of SCE/cell, with each dose group significantly higher than the negative control. For these reasons, these data seem to be convincing

⁶ The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

although obtained on a small (3) number of animals/group. The relevance of SCE in spermatogonia as an indicator of heritable genetic damage is limited.

In the same paper Tian et al. (Tian et al., 1992) reported the induction of micronuclei in early sperm cells. This test measures the induction of DNA lesions in preleptotene spermatocytes that can lead to breaks and fragments several days later, at the first or second meiotic division. The test has not been standardised and validated for routine regulatory application, but has been conducted by more than one laboratory in the world with consistent results. The study seems adequately performed. Staining with Giemsa is not optimal and does not allow to distinguish among phases of spermatid differentiation as recommended by the guidelines (Russo, 2000). However, this drawback could hardly produce an overestimation of the effect, more likely, if any, an underestimation.

4-Hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] and *2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone* [FL-no: 13.084]

Reverse mutations were also observed in *S. typhimurium* TA100, but not TA98 with 4-hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] and with 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084]. The other strains were not tested. The same substances could induce single strand breaks in purified DNA. With 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] also induction of micronuclei in peripheral erythrocytes was observed in two valid *in vivo* assays.

Mechanistic Data

For the substances in subgroup 4.4b also mechanistic studies were carried out with [FL-no: 13.010, 13.084 and 13.085], all of which were considered valid. These substances were identified as Maillard reaction products in soy sauce. When the substance [FL-no: 13.085] was incubated with super coiled pBR 322 plasmid DNA, single strand breaks were observed at pH 4.4, but not at pH 7.4. When a spin trap was also present, formation of hydroxy radicals together with a carbon-centred radical could be demonstrated. Subsequent addition of superoxide dismutase and catalase inhibited the DNA breaking showing involvement of hydrogen peroxide. Potassium iodide, mannitol, sodium azide, and ethanol were also inhibitory to the DNA breaking showing involvement of hydroxy radicals. Spin trapping agents and thiol compounds and metal chelators also effectively inhibited the breaking of DNA (Hiramoto et al., 1996a). Similar studies were carried out with [FL-no: 13.010 and 13.084] with the same results and it was also demonstrated that these substances are capable of reducing Fe^{3+} at neutral or alkaline pH (Li et al., 1998).

For validation and study results see Table 2.1 and 2.2.

Conclusion on Genotoxicity and Carcinogenicity - Text Taken from FGE.220 (EFSA, 2009ae)

Apart from the negative predictions for the substances in the DTU-NFI MultiCASE model for the Ames test, the (Q)SAR models do not seem to generate a reliable and reproducible pattern of predictions on the genotoxicity for the substances in this FGE.

With several substances in subgroup 4.4b [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176], indications have been obtained in *in vitro* studies that the genetic damage they cause is related to the generation of reactive oxygen species as a result of redox cycling in combination with metal ions present in the media. The valid positive *in vivo* data were obtained with high dose levels that may be anticipated to have exhausted the anti-oxidant capacity of the target cells. This, in combination with the absence of carcinogenicity observed in a valid carcinogenicity study in rats with one of the substances [FL-no: 13.010], which was tested positive in the genotoxicity assays, takes away a concern for genotoxic events resulting in carcinogenicity in somatic cells.

For two of the studies in which genotoxic effects were observed in germ cells *in vivo* the studies had limited validity and/or address endpoints that may have limited relevance for the assessment of genotoxic potential. The Panel noted that a positive result was obtained in a micronucleus study in

early sperm cells. However, a micronucleus test does not discriminate between aneuploidy and chromosomal breakage. The observed effects in the germ cells could be the result of the malsegregation of chromosomes which is generally considered a thresholded event. They may alternatively be the result of the (thresholded) generation of reactive oxygen species.

Conclusion – Text Taken from FGE.220 (EFSA, 2009ae)

For the substances in subgroup 4.4b [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176], evidence for genotoxicity was obtained *in vitro* and *in vivo*. Evidence is available from *in vitro* studies that the genotoxicity of the candidate substances in this subgroup may be caused by indirect (threshold) mechanisms of action (in particular generation of reactive oxygen species). The concern for carcinogenicity is alleviated, since one of the substances, for which positive genotoxicity data in mice were obtained, was not carcinogenic in a valid chronic assay in rats. Therefore, no further genotoxicity tests in somatic cells are required. However, some evidence was also available that this substance might elicit genotoxic effects in germ cells, which theoretically may result in reduced reproductive capacity or in inheritable genetic damage. Reduced reproductive capacity and inheritable genetic damage are toxicological endpoints which differ from carcinogenicity and therefore, the negative results for the carcinogenicity study cannot be used to overrule this concern. Also it is not clear if (and if so to what extent) the threshold mechanism mentioned above would be relevant for genotoxic effects in the germ cells. Therefore, the Panel concluded that presently these five substances cannot be evaluated through the Procedure.

The Panel recognised that the studies which provided indications for germ cell genotoxicity are of limited validity. For that reason a robust GLP-controlled cytogenetic investigation in mouse spermatocytes according to the OECD Guideline 483 is requested.

Additional Data Submitted by Industry

In response to the EFSA request in FGE.220 of a cytogenetic study in mouse spermatocytes (OECD TG 483), Industry has submitted the following data:

- 2-Year carcinogenicity bioassay in rats with a substance coded ST 07 C99 (this is the study on [FL-no: 13.010] by Kelly & Bolte, 2003);
- Oral male fertility study of FURANEOL = 4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] (test article code ST17C07) in rats (Sloter, 2008);
- Oral micronucleus assay in bone marrow cells of the mouse with NEOFURANEOL (no identification of this substance is available) (Honarvar, 2008b);
- Mouse lymphoma (TK) specific locus mutation assay with compound 0478/1 (Ross and Harris, 1979a).

Evaluation of Additional Data

The Panel noted that among the studies submitted by Industry, only the rat fertility study, which includes also the analysis of dominant lethals, is considered relevant for the specific EFSA request.

The 2-year carcinogenicity bioassay in rats by Kelly and Bolte (Kelly and Bolte, 2003) was already evaluated by the Panel in the previous version of FGE.220. It was considered as a valid, negative study, however not relevant for the evaluation of possibly inheritable damage. Also the mouse bone marrow micronucleus assay with neofuraneol (Honarvar, 2008b) and the *in vitro* mouse lymphoma TK assay (Ross and Harris, 1979a) are considered not relevant to clear the concern for possible inheritable damage. Furthermore, an adequate identification of the test substance Neofuraneol was not possible, due to incomplete reporting. For these reasons these three studies will not be further considered in this section.

Oral Male Fertility Study of 4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] in Rats (Sloter, 2008)

The objective of this study, performed according to ICH Guideline 4.1.1 (ICH, 1996) under GLP, was to determine the potential effects of 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] on mating, fertility and gonadal function in male rats with two separate mating trials. 4-Hydroxy-2,5-dimethylfuran-3(2H)-one was administered by gavage once daily to three groups of 25 male Crl:CD(SD) rats. Dosage levels were 100, 500 and 1000 mg/kg bw/day. A concurrent control group of 25 males received the vehicle (propylene glycol) on a comparable regimen. The first mating (Phase I), following 2 weeks of male administration, using untreated females, was conducted to detect potential elicitation of early genotoxic effects on the embryo with reduced risk of test-article related deficiencies in mating or fertility. The second mating (Phase II), following 9 weeks of male dose administration, was conducted following male exposure throughout a complete spermatogenic cycle using a second set of untreated females.

There was no test-article related mortality noted in this study. A slightly lower mean body-weight gain was noted in the 1000 mg/kg/day group when evaluated for the overall treatment period. No test-article related effects on male reproductive performance were observed at 100, 500 and 1000 mg/kg/day when males were mated with Phase I or Phase II females. In particular, there were no effects on spermatogenic endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility and morphology, reproductive organs or macroscopic findings) at any of the doses tested. The mean percentage of sperm with abnormal morphology (separated head and flagellum) was slightly higher in the 500 and 1000 mg/kg/day groups; however, this was primarily attributed to a single male in the respective groups and therefore not considered test-article related. The number of females mated and the number of pregnant females was comparable to controls. Uterine examination was performed for both Phase I and Phase II females. The analysis of embryonic data (corpora lutea, implantation sites, viable embryos, dead embryos, early resorptions, late resorptions, total resorptions, post- and pre-implantation losses) did not reveal dominant lethal effects. The study does not indicate a potential of 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] to affect male fertility. This study can be considered to be equivalent to an OECD 478 Dominant Lethal assay. The dominant lethal assay has been recommended as a follow-up study in case of positive results in the OECD TG 483 (Eastmond et al., 2009). On this basis the Panel considers it acceptable to substitute the requested study according to OECD Guideline 483 with the Dominant Lethal test.

Conclusion on Additional Data

The results of a valid rat fertility and dominant lethal study have shown that 4-hydroxy-2,5-dimethylfuran-3(2H)-one is unable to induce both adverse effects on male rat reproductive capacity and dominant lethality. On this basis the Panel concludes that for this substance there is no concern for its potential to induce heritable genetic damage or adverse effects on male reproductive capacity. Accordingly the substances in subgroup 4.4b of FGE.19 [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] can be evaluated using the Procedure.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by EFSA see Table 2.1, 2.2 and 2.3.

3.3. EFSA Considerations

The Panel concluded that the positive results observed in the genotoxicity studies on the substances in this group were due to the production of reactive oxygen species, potentiated by the presence of metals in the cell medium. The resulting DNA damage is only observed once the cell antioxidant capacity has been exhausted. This effect is unlikely to occur at the low levels used to flavour foods. A concern for a potential to elicit heritable genetic damage was alleviated by the results of a valid rat fertility and dominant lethal study which show that 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] is unable to induce both adverse effects on male rat reproductive capacity and dominant lethality. On this basis the Panel concludes that for this substance and structurally related substances there is no concern for its potential to induce heritable genetic damage or adverse effects on male reproductive capacity. Accordingly, available genotoxicity data presented in this FGE do not preclude the

evaluation of the substances in subgroup 4.4b of FGE.19 [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] using the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to Five Furanone Derivatives by the JECFA

According to the JECFA four substances belong to structural class II and one substance [FL-no: 13.176] to structural class III using the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

One substance from the 65th and 69th meetings (JECFA, 2006b; JECFA, 2006d; JECFA, 2009a; JECFA, 2009c)

For this substance [FL-no: 13.176] the JECFA concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group because of the unresolved toxicological concerns relating to the epoxidation and opening of the furan ring.

Four substances from the 63rd meeting (JECFA, 2005c; JECFA, 2006a)

The JECFA concluded three [FL-no: 13.084, 13.085 and 13.099] of these four furanone derivatives at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for the substances are below the thresholds for their structural classes (step A3).

The last substance [FL-no: 13.010] has intake above the threshold of concern for the corresponding structural class (step A3); it is not endogenous (step A4), but an adequate NOAEL of 200 mg/kg bw/day is available from the two year carcinogenicity study by Kelly and Bolte, 2003 (Kelly and Bolte, 2003) that provides an adequate margin of safety (2.7×10^3) from the exposure as a flavouring substance (step A5).

In conclusion, the JECFA evaluated the four substances to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the furanone derivatives are summarised in Table 3.1: Summary of Safety Evaluation of Furanone Derivatives.

4.2. EFSA Considerations

The Panel agrees only with the application of the Procedure as performed by the JECFA for one substance [FL-no: 13.010].

The JECFA stated that the three substances [FL-no: 13.084, 13.085 and 13.099] belong to structural class II, but as data on natural occurrence are not available for these three substances, the Panel disagree with this and allocate these three substances to structural class III.

With regards to the substance [FL-no 13.176], the Panel disagreed with the conclusions by the JECFA. The JECFA put the assessment of this structural class III substance on hold because of concerns for a potential for epoxidation and ring opening which may lead to genotoxic activity. The Panel regarded this substance to be sufficiently structurally related to the other substances in this group. In FGE.220Rev1, the Panel concluded that data available on substances structurally related to all the candidate substances in the present FGE demonstrate that these substances do not pose a concern for genotoxicity and can be evaluated through the Procedure.

Since the exposure estimates (MSDI) for Europe for two substances [FL-no: 13.085 and 13.176] is below the threshold for structural class III (i.e. 90 µg/person/day), at step A3 of the Procedure it is concluded that these two substances do not pose a safety concern when used as a flavouring substance in food.

For the remaining two substances [FL-no: 13.084 and 13.099] the exposure estimates (MSDI) for Europe are above the threshold for structural class III. Since these substances are not endogenous their evaluation proceeds to step A5 of the Procedure. At this step the respective exposure estimates (203 and 400 µg/capita/day) can be compared to the NOAEL of 200 mg/kg bw/day for [FL-no 13.010], which is available from the two year carcinogenicity study by Kelly and Bolte (Kelly and Bolte, 2003). This NOAEL is based on decreases in mean body weights and body weight gains of male and female rats exposed to 400 mg 4-hydroxy-2,5-dimethyl-3(2H)-furanone/kg bw/day compared to those of the controls in the last part of the study. No neoplasms or non-neoplastic lesions were attributed to exposure to 4-hydroxy-5-dimethyl-3(2H)-furanone. Adequate margins of safety of 5.9×10^4 and 3.0×10^4 can be calculated for [FL no: 13.084] and [FL-no: 13.099], respectively, from which it is concluded that these two substances do not pose a safety concern when used as a flavouring substance in food.

Thus the Panel concluded that there is no safety concern for any of the candidate substances in this FGE based on the MSDI approach.

5. Conclusion

This consideration deals with five flavouring substances [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176], which belong to a group of 18 tetrahydrofuran and furanone derivatives and a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulphides, disulphides and ethers evaluated by the JECFA at the 63rd, 65th and 69th meetings.

The Panel agrees with the application of the Procedure as performed by the JECFA for only one substance [FL-no: 13.010] considered in this FGE. The JECFA stated that the three substances [FL-no: 13.084, 13.085 and 13.099] belong to structural class II, but as data on natural occurrence are not available for these three substances, the Panel disagree with this and allocate these three substances to structural class III. In addition, for the substance [FL-no: 13.176] the JECFA concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied because of the unresolved toxicological concerns relating to the epoxidation and opening of the furan ring. The Panel disagreed with the conclusions by the JECFA and regarded this substance to be sufficiently structurally related to the other substances in this group.

The Panel concluded that there is no safety concern for the five substances in this FGE, based on the MSDI approach.

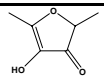
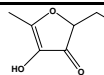
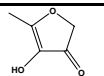
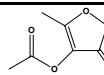
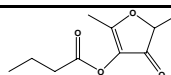
For all five substances evaluated through the Procedure use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the five JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all JECFA evaluated substances.

Thus, for all five substances [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] the Panel concluded that they would be of “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Furanone Derivatives (JECFA, 2005b; JECFA, 2005d)

FL-no JECFA- no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
13.010 1446	4-Hydroxy-2,5-dimethylfuran-3(2H)-one		3174 536 3658-77-3	Solid C ₆ H ₈ O ₃ 128.13	Insoluble Soluble	n.a. 78-80 IR NMR MS 98 %	n.a. n.a.	Racemate (EFFA, 2012d).
13.084 1449	2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone		3623 27538-09-6	Liquid C ₇ H ₁₀ O ₃ 142.15	Soluble Soluble	103 (20 hPa) IR NMR 96 %	1.509-1.514 1.133-1.143	Racemate (EFFA, 2012d).
13.085 1450	4-Hydroxy-5-methylfuran-3(2H)-one		3635 11785 19322-27-1	Solid C ₅ H ₆ O ₃ 114.10	Soluble Soluble	n.a. 126-133 NMR 97 %	n.a. n.a.	
13.099 1456	4-Acetoxy-2,5-dimethylfuran-3(2H)-one		3797 4166-20-5	Liquid C ₈ H ₁₀ O ₄ 170.17	Slightly soluble Soluble	243 IR NMR MS 95 %	1.476-1.480 1.159-1.167	According to JECFA: Min. assay value is "85%" and secondary components "4-hydroxy-2,5-dimethyl-3(2H)-furanone". Racemate (EFFA, 2012d).
13.176 1519	Furaneyl butyrate		3970 114099-96-6	Liquid C ₁₀ H ₁₄ O ₄ 198.22	Insoluble Soluble	287 NMR 95 %	1.467-1.473 1.095-1.103	According to JECFA: Min. assay value is "93 %" and secondary components "4-Hydroxy-2,5-dimethylfuran-3-one, butyric acid" Register name to be changed to 4-Butyrox-2,5-dimethyl-3(2H)-furanone (EFFA, 2012d) CASrn 114099-96-6 to be introduced in the Register (EFFA, 2012d) Racemate (EFFA, 2012d).

- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95 % ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: GENOTOXICITY (*in vitro*) EFSA / FGE.220Rev1 (EFSA, 2011a)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments ^e
4-Hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Reversed mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA100 and TA98	10.0, 33.3, 100.0, 333.3, 1000, 2000, 3300, 4000, 6000, 8000 µg/plate	Positive ^{a, b}	(Gilroy et al., 1978)	Valid. Unpublished non-GLP study. The report contains sufficient details. Result is considered valid.
	Reversed mutation	<i>S. typhimurium</i> TA100 and TA98	0 - 10000 µg/plate	Positive ^{a, b}	(Hiramoto et al., 1996b)	Valid. Positive in TA100 (+/- S9); negative in TA98 (+/- S9).
	Reversed mutation	<i>S. typhimurium</i> TA100, TA102, TA98 and TA97	500 - 4000 µg/plate	Positive ^{a, c}	(Xing et al., 1988)	Limited validity. No methodological details, but stated to be performed according to (Maron and Ames, 1983). Some errors reduce the trustworthiness of the paper.
	Reversed mutation	<i>E. coli</i> WP2 uvrA ⁻	10.0, 33.3, 100.0, 333.3, 1000, 3300 µg/plate	Negative	(Gilroy et al., 1978)	Valid. Unpublished non-GLP study. The report contains sufficient details. Result is considered valid.
	DNA damage	<i>B. subtilis</i> H17 (Rec ⁺) and M45 (Rec ⁻)	20, 40, 60, 80, 120 µg/disc	Positive	(Xing et al., 1988)	Validity cannot be evaluated (Test system with low predictive value for genotoxicity). No methodological details, but stated to be performed according to (Kada et al., 1972).
	DNA strand breaks	pBR322 DNA	2.6 - 780 µmol/l (0.3 - 100 mg/l)	Positive	(Hiramoto et al., 1996b)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.
4-Hydroxy-5-methylfuran-3(2H)-one [13.085]	Reversed mutation	<i>S. typhimurium</i> TA100 and TA98	0 - 5000 µg/plate	Positive ^{a, b}	(Hiramoto et al., 1996a)	Limited validity. Limited due to uncertainty of test substance. Positive in TA100 (+/- S9); negative in TA98 (+/- S9).
	DNA strand breaks	pBR322 DNA	0 - 900 µmol/l (0 - 103mg/l)	Positive ^{a, d}	(Hiramoto et al., 1996a)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.
2,5-Dimethyl-3(2H)-furanone [13.119]	Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100 and TA102	0 - 5000 µg/plate	Negative	(RCC - CCR, 2007)	Valid. According to current guidelines.
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone [13.084]	Reversed mutation	<i>S. typhimurium</i> TA100 and TA98	0 - 10000 µg/plate	Positive ^{a, b}	(Li et al., 1998)	Valid. Positive with and without S9 in TA100; negative in TA98 (+/- S9).
	DNA strand breaks	pBR322 DNA	0 - 2000 µM	Positive ^d	(Li et al., 1998)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.

a: With and without metabolic activation provided by S9 (9000 x g supernatant from rodent liver).

b: Positive results only observed in TA100.

- c: Positive results in all strains at the highest dose tested.
- d: Only positive without inhibitors of redox cycling and ROS scavengers.
- e: Validity of genotoxicity studies:
 - Valid.
 - Limited validity (e.g. if certain aspects are not in accordance with OECD Guidelines or current standards and / or limited documentation).
 - Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
 - Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

Table 2.2: GENOTOXICITY (*in vivo*) EFSA / FGE.220Rev1 (EFSA, 2011a)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments ^a
4-Hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Micronucleus formation	Mouse, bone marrow	Not stated	0, 186, 232 or 309 mg/kg bw	Positive	(Xing et al., 1988)	Limited validity. Important data not given. Reference to methodological description could not be traced.
	Chromosomal aberration	Mouse spermatocytes	Not stated	0, 232, 464 or 928 mg/kg bw	Positive	(Xing et al., 1988))	Limited validity. Important data not given. Reference to methodological description could not be traced. Predominant aberration; malsegregation of chromosomes.
	Sister chromatid exchange	Mouse, bone marrow	Intra-abdominal injection	0, 185, 232, 303 mg/kg	Positive	(Xing et al., 1988)	Validity cannot be assessed. Dose-related increase; statistically significant at all dose levels, but max increase < 2-fold. Effect not adequately specified; very intense exposure to BrdU. Non-validated protocol. Relevance for the evaluation of genotoxicity questionable.
	Sister chromatid exchange	Mouse spermatocytes	Oral (gavage)	200, 400 or 800 mg/kg bw	Positive	(Tian et al., 1992)	Limited validity. Relevance for the evaluation of genotoxicity questionable; non-validated test protocol.
	Micronucleus formation	Mouse early sperm cells	Oral (gavage)	200, 400 or 800 mg/kg bw	Positive	(Tian et al., 1992)	Limited validity Non-validated test protocol.
	Micronucleus formation	Mouse peripheral blood cells	Gavage	1000, 2000 3000 mg/kg bw	Positive	(Hiramoto et al., 1998)	Valid.
	Micronucleus formation	Male mice peripheral erythrocytes	I.p.	500, 1000, 1500mg/kg bw	Positive	(Hiramoto et al., 1996b)	Valid.
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone [13.084]	Micronucleus formation	Mouse peripheral blood cells	Gavage	0, 1000, 2000 and 3000 mg/kg bw	Positive	(Hiramoto et al., 1998)	Valid.
	Micronucleus formation	Male mice peripheral erythrocytes	I.p.	0, 500, 1000 and 1500 mg/kg bw	Positive	(Li et al., 1998)	Valid.

a: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

Table 2.3: ADDITIONAL GENOTOXICITY DATA (*in vitro* and *in vivo*) EFSA / FGE.220Rev1 (EFSA, 2011a)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments ^a
4-Hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	-	111, 167, 250, 375 and 750 µg/ml	Negative both with and without S9	(Ross and Harris, 1979a)	Limited validity. Study not performed according to current guideline. Too short treatment and no differentiation between small and large colonies.
	Dominant lethal assay in a rat fertility study	Dominant lethals in CrI:CD(SD) male rats (25/group)	Oral gavage	100, 500 and 1000 mg/kg bw/day for 2 weeks (Phase I) and 9 weeks (Phase II)	No increase of dominant lethal effects	(Sloter, 2008)	Valid GLP study in accordance with ICH Guideline 4.1.1.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of Furanone Derivatives (JECFA, 2006a; JECFA, 2009a)

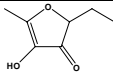

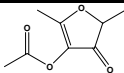

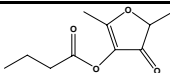
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.084 1449	2-Ethyl-4-hydroxy-5-methyl-3(2H)- furanone		203 13	Class II A3: Intake below threshold	4)	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.085 1450	4-Hydroxy-5-methylfuran-3(2H)- one		47.8 0.07	Class II A3: Intake below threshold	4)	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.099 1456	4-Acetoxy-2,5-dimethylfuran- 3(2H)-one		400 8	Class II A3: Intake below threshold	4)	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.010 1446	4-Hydroxy-2,5-dimethylfuran- 3(2H)-one		4483 5203	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.176 1519	Furanyl butyrate		4.2 4	Class III No evaluation		Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. Register name to be changed to 4-Butyroxyl-2,5- dimethyl-3(2H)-furanone

Table 3.1: Summary of Safety Evaluation of Furanone Derivatives (JECFA, 2006a; JECFA, 2009a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
							(EFSA, 2012d).

- 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = $\mu\text{g/capita/day}$.
- 2) Thresholds of concern: Class I = 1800 $\mu\text{g/person/day}$, Class II = 540 $\mu\text{g/person/day}$, Class III = 90 $\mu\text{g/person/day}$.
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.
- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.

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ABBREVIATIONS

BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
ID	Identity
I.p.	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
(Q)SAR	(Quantitative) Structure-Activity Relationship

SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organisation